

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

EPA MRID No. 473723-39

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|--------------------------|-----------------|---------------|
| Data Requirement: | PMRA Data Code | {.....} |
| | EPA DP Barcode | 353315 |
| | OECD Data Point | {.....} |
| | EPA MRID | 473723-39 |
| | EPA Guideline | Non-guideline |

| | | |
|-----------------------|---|-----------------------------------|
| Test material: | [¹⁴ C]AEC656948 | Radiochemical Purity: >99% |
| | Unlabeled AEC656948 | Purity: 94.7% |
| Common name | Fluopyram | |
| | Chemical name: | |
| | IUPAC: N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl]- α,α,α -trifluoro-o-toluamide | |
| | CAS: N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)benzamide | |
| | CAS No.: 658066-35-4 | |
| | Synonyms: None reported | |

Reference/Submission No.: {.....}

| | | |
|--------------------------|---------|------------|
| Company Code | {.....} | [For PMRA] |
| Active Code | {.....} | [For PMRA] |
| Use Site Category | {.....} | [For PMRA] |
| EPA PC Code | 080302 | |

CITATION: Putt, A.E. 2008. AEC656948 – Life-Cycle Toxicity Test Exposing Midges (*Chironomus tentans*) to a Test Substance Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods. Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory Study No. 13798.6212. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated April 12, 2007 and submitted February 29, 2008.

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EXECUTIVE SUMMARY:

The 54-day life-cycle toxicity of AEC656948 (fluopyram) to the freshwater dipteran midge *Chironomus tentans* was studied under static renewal conditions. First instar larvae were exposed to AEC656948 at nominal sediment concentrations of 0 (negative and solvent controls), 7.5, 15, 30, 60, and 120 mg ai/kg dw sediment. TWA sediment concentrations were <0.051 (controls), 6.2, 13, 26, 48, and 96 mg total radioactive residues (TRR)/kg dw sediment, respectively. TWA pore water concentrations were <0.0097 (controls), 0.66, 1.9, 3.8, 8.8, and 18 mg TRR/L, respectively; and TWA overlying water concentrations were <0.0029 (controls), 0.032, 0.047, 0.14, 0.29, and 0.88 mg TRR/L, respectively. The 20-day LC/EC₅₀ exceeded the highest concentration level for both larval survival and growth (day 20). The 54-day NOAEC was 26 mg TRR/kg sediment (corresponding with 3.8 mg TRR/L pore water and 0.14 mg TRR/L overlying water), based upon statistically-significant reductions in larval survival (day 20) and percent emergence (day 54).

The most sensitive endpoints were larval survival (assessed on day 20) and percent emergence; these endpoints were statistically-reduced at the 48 and 96 mg TRR/kg sediment levels. Midge growth (assessed on day 20) and development rate for both sexes were statistically-reduced at the 96 mg TRR/kg sediment level. No treatment-related effect on time to death for mated adults, number of eggs per female, or percent hatch of egg masses were indicated.

This study is scientifically sound/unsound and {does or does not} satisfy the guideline requirement for a life-cycle toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age: first-instar larvae, 3 hours post-hatch

Test Type (Flow-through, Static, Static Renewal): Static renewal

Endpoint(s) affected: larval survival (day 20), larval growth (day 20), percent emergence, and development rate

Most sensitive endpoint(s): larval survival (day 20) and percent emergence

Based on TWA sediment concentrations (total radioactive residues):

LC₅₀ survival (day 20): >96 mg/kg

NOAEC (survival): 26 mg/kg

LOAEC (survival): 48 mg/kg

EC₅₀ dry weight (day 20): >96 mg/kg

NOAEC (dry weight): 48 mg/kg

LOAEC (dry weight): 96 mg/kg

NOAEC (percent emergence): 26 mg/kg

LOAEC (percent emergence): 48 mg/kg

NOAEC (combined sex development rate): 48 mg/kg

LOAEC (combined sex development rate): 96 mg/kg

NOAEC (time to death for mated adults): 98 mg/kg

LOAEC (time to death for mated adults): >98 mg/kg

NOAEC (number of eggs per female): 98 mg/kg

LOAEC (number of eggs per female): >98 mg/kg

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NOAEC (percent hatch of egg masses): 98 mg/kg
LOAEC (percent hatch of egg masses): >98 mg/kg

Based on TWA pore water concentrations (total radioactive residues):

LC₅₀ survival (day 20): >18 mg/L

NOAEC (survival): 3.8 mg/L

LOAEC (survival): 8.8 mg/L

EC₅₀ dry weight (day 20): >18 mg/L

NOAEC (dry weight): 8.8 mg/L

LOAEC (dry weight): 18 mg/L

NOAEC (percent emergence): 3.8 mg/L

LOAEC (percent emergence): 8.8 mg/L

NOAEC (combined sex development rate): 8.8 mg/L

LOAEC (combined sex development rate): 18 mg/L

NOAEC (time to death for mated adults): 18 mg/L

LOAEC (time to death for mated adults): >18 mg/L

NOAEC (number of eggs per female): 18 mg/L

LOAEC (number of eggs per female): >18 mg/L

NOAEC (percent hatch of egg masses): 18 mg/L

LOAEC (percent hatch of egg masses): >18 mg/L

Based on TWA overlying water concentrations (total radioactive residues):

LC₅₀ survival (day 20): >0.88 mg/L

NOAEC (survival): 0.14 mg/L

LOAEC (survival): 0.29 mg/L

EC₅₀ dry weight (day 20): >0.88 mg/L

NOAEC (dry weight): 0.29 mg/L

LOAEC (dry weight): 0.88 mg/L

NOAEC (percent emergence): 0.14 mg/L

LOAEC (percent emergence): 0.29 mg/L

NOAEC (combined sex development rate): 0.29 mg/L

LOAEC (combined sex development rate): 0.88 mg/L

NOAEC (time to death for mated adults): 0.88 mg/L

LOAEC (time to death for mated adults): >0.88 mg/L

NOAEC (number of eggs per female): 0.88 mg/L

LOAEC (number of eggs per female): >0.88 mg/L

NOAEC (percent hatch of egg masses): 0.88 mg/L

LOAEC (percent hatch of egg masses): >0.88 mg/L

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I. REPORTED MATERIALS AND METHODS

GUIDELINE FOLLOWED: U.S. EPA “Methods for Assessing the Chronic Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, 2nd Ed. Test Method 100.5 March 2000. EPA/600/R-99/064.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was conducted in compliance with all pertinent U.S. EPA GLP Regulations (40 CFR, Part 160) with the following exception: routine water, sediment and food contaminant screen analyses for pesticides, PCBs and toxic metals. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results.

A. REPORTED MATERIALS:

1. Test Material

Table 1: Test material(s).

| Parameter | Details |
|----------------------|---|
| Radiolabeled | |
| Name | [pyridyl-2,6- ¹⁴ C]AEC656948 (fluopyram) |
| Description | Not reported |
| Lot No./Batch No. | BECH 2168 (Sample ID) |
| Radiochemical Purity | >99% |
| Specific Activity | 3.85 MBq/mg |
| Storage conditions | In a freezer (-70 to -90°C) in original container |
| Non-radiolabeled | |
| Name | AEC656948 (fluopyram) |
| Description | Not reported |
| Lot No./Batch No. | 08528/0002 |

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| Parameter | Details |
|---------------------------------|--|
| Purity | 94.7% |
| Storage conditions | Room temperature in original container in the dark |
| Stability under test conditions | Not assessed |

Table 2: Physicochemical properties of fluopyram.

| Parameter | Values |
|--------------------------|--------------------------|
| Water solubility at 20°C | 16 mg/L (MRID 473723-40) |
| Vapor pressure | Not reported |
| UV absorption | Not reported |
| pKa | Not reported |
| Kow | Not reported |

2. Test organism:

Table 3: Test organism.

| Parameter | Details |
|-----------|---|
| Species: | Dipteran midge, <i>Chironomus tentans</i> |
| Age/Size: | First instar, 3-hours old |
| Source: | Aquatic BioSystems, Fort Collins, CO |

B. REPORTED STUDY DESIGN:

1. Experimental Conditions

a. Preliminary Studies: A 43-day range-finding test was conducted with non-radiolabeled AEC656948 (purity of 94.7%) at nominal treatment levels of 0 (negative and solvent controls), 0.0095, 0.095, 0.95, 9.5, and 95 mg ai/kg sediment. Eight replicate vessels containing 12 midge larvae (<24 hours old) each were exposed; otherwise, methods followed those described for the definitive study. Nominal levels for the definitive study were selected based on the results of the preliminary study (summarized in the following table).

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Table 4: Preliminary study results.

| Nominal Treatment Level (mg ai/kg sediment) | % Survival Day 20 | Mean Dry Weight per Midge, mg | Mean % Emergence | Development Rate | No. Eggs per Female | Mean % Hatch |
|---|-------------------|-------------------------------|------------------|------------------|---------------------|--------------|
| Negative control | 78 | 1.38 | 68 | 0.0409 | 1102 | 97 |
| Solvent control | 83 | 1.72 | 82 | 0.0407 | 1077 | 96 |
| 0.0095 | 86 | 2.30 | 87 | 0.0419 | 1287 | 91 |
| 0.095 | 83 | 2.00 | 70 | 0.0412 | 1299 | 94 |
| 0.95 | 92 | 1.99 | 58 | 0.0417 | 1193 | 96 |
| 9.5 | 75 | 2.70 | 72 | 0.0428 | 1548 | 98 |
| 95 | 64 | 1.83 | 32* | 0.0356 | 1018 | 99 |

*Statistically different compared to the control data.

b. Definitive Study

Table 5: Experimental Parameters

| Parameter | Details |
|--|---|
| <u>Parental acclimation:</u> Period: Culture conditions: Feeding: Health (any mortality observed): | N/A Egg masses were placed in 270-mL crystallizing dishes containing approximately 150 mL of laboratory well water. The egg masses were observed daily until hatch, and were then transferred into clean laboratory water to facilitate the larvae to leave the egg mass. Midge larvae were fed a finely-ground flaked fish food suspension (4.0 mg/mL) Not reported |
| Type of test system | Static renewal |
| Duration of the test | 54 days |

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| Parameter | Details |
|---------------------------|---|
| Test water | Laboratory well water having a total hardness and total alkalinity of 36 to 44 and 18 to 23 mg/L as CaCO ₃ , respectively, a pH range of 6.3 to 7.0, and a specific conductivity of 160 to 210 µmhos/cm. |
| Test sediment | <p>Formulated (artificial) sediment (Batch Nos. 110607 and 112807) was prepared by blending the following dry components in a large-scale laboratory mixer:</p> <p>1.2 kg (6%) peat moss 4.0 kg (20%) kaolin clay 7.4 kg (37%) fine sand 7.4 kg (37%) coarse sand</p> <p>The artificial sediment used in this study (Batch No. 11307) was a composite of the two prepared batches.</p> |
| Sediment characterization | <p>Particle size: 75% sand, 4% silt, and 21% clay Percent organic carbon: 2.2% pH: 6.4 Percent water content (1/3 bar): 14.7%</p> |
| Dosing Stock Solutions | <p>A primary radiolabeled stock solution was prepared in acetone at 0.265 mg ai/mL. The radiolabeled stock solution was stored in a freezer (-70 to -90°C) in an amber-colored bottle.</p> <p>A primary non-radiolabeled stock solution was prepared in acetone at 45 mg ai/mL. The solution was dark amber in color with no visible un-dissolved test material present.</p> <p>Using a combination of the two primary stock solutions and additional acetone, five individual dosing stock solutions were prepared. The dosing stock solutions ranged from clear and light yellow in color to amber-colored.</p> |

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| Parameter | Details |
|---|--|
| Sediment spiking | <p>The study author reported that a jar-rolling technique was used to apply the test substance to the sediment. A 9-mL volume of each stock solution was applied to 0.050 kg of fine silica sand and the solvent was allowed to evaporate off for 60 minutes. The dry sand was then added to 3.0 kg of wet sediment (2.4954 kg dw). Each jar was then rolled for 4 hours at room temperature at approx. 15 rpm. The jars were stored upright at 2 to 8°C overnight prior to conditioning.</p> |
| Solvent concentration | <p>Acetone, 9 mL per 2.5454 kg total mass of sediment (dry weight). The acetone was allowed to evaporate during the mixing procedure.</p> <p>Both solvent control and negative control groups were included in the study.</p> |
| Sediment conditioning | <p>The treated sediments were allowed to equilibrate for a 14-day period in the refrigerator. Once a week and prior to addition to the exposure vessels (day -1), the jars were mixed on the rolling mill for an additional 2 hours at room temperature to ensure the sediment was homogeneous.</p> |
| Sediment and overlying water into test chambers | <p>Test systems were established 2 days prior to the addition of chironomid larvae (day -2). Overlying water was gently added.</p> <p>300-mL glass vessels containing 100 mL (<i>ca.</i> 4.0-cm layer) of sediment and 175 mL of overlying water (275 mL total volume). Test vessels were covered with 40-mesh Nitex® screen for drainage. On Day 17, emergence traps were placed over the test vessels to trap emergent flies for the remainder of the test.</p> <p>20 replicates were prepared for each test concentration and control: 12 replicates were used to evaluate the biological response (four for survival and growth on Day 20 and eight for monitoring emergence); four were established on Day 10 for production of auxiliary males during the emergence phase; and the remaining four replicates were maintained for the purpose of chemical analysis.</p> |

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| Parameter | Details |
|---|---|
| Renewal of overlying water | The overlying water was replaced twice daily using an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least once daily for proper functioning. |
| Aeration | None reported |
| Adult midge collection for reproduction | <p>Beginning on Day 18 and daily thereafter, emerged male and female midge were collected from the emergence traps, placed in reproductive/oviposit chambers, and held individually until sufficient numbers were available to pair male:female flies.</p> <p>Auxiliary males were used to mate female flies towards the end of the female emergence period (as male flies emerge typically 5 to 7 days prior to female flies).</p> <p>Each male could be used for mating with (one or more) females from corresponding treatment levels for up to 5 days, and males from different replicates (within the same treatment level) were paired with females where no males had emerged.</p> |
| Reproductive/oviposit chambers | Plexiglass tubes (3.5-cm length, i.d. 6 cm) covered on the top with wide-mesh Nitex® screen and placed on a 100 x 20-mm Petri dish. With fly addition, 50 mL of laboratory well water was added to the chambers. |
| Water quality monitoring | <p><u>Overlying water:</u> DO, temperature, and pH were measured in each (biological) replicate vessel on Days -1, 0, 10, 20, and 54, and temperature and DO were measured daily in alternating (supplemental) test vessels from each level. Temperature was also continuously monitored in an auxiliary vessel. Total hardness, alkalinity, specific conductance, and ammonia concentrations were measured in a composite sample from all replicates on Days -1, 0, 10, 20, and 54.</p> <p><u>Pore water:</u> not analyzed</p> |
| Dissolved oxygen | 2.5 to 8.6 mg/L |

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| Parameter | Details |
|--|---|
| Temperature | 21 to 25°C |
| pH | 6.2 to 7.2 |
| Specific conductance | 220 to 270 µmhos/cm |
| Hardness | 32 to 52 mg/L as CaCO ₃ |
| Alkalinity | 18 to 30 mg/L as CaCO ₃ |
| Ammonia (as N) | ≤0.10 to 2.8 mg/L |
| Photoperiod | 16 hours light, 8 hours dark (400 to 630 lux) |
| Feeding | Finely-ground flaked fish food suspension (4.0 mg/mL) Once daily, at a rate of 1.5 mL per test vessel |
| <u>Treatment Concentrations:</u> Nominal sediment: Mean-measured sediment: | 0 (negative and solvent controls), 7.5, 15, 30, 60, and 120 mg ai/kg dw sediment (adjusted for purity) <0.051 (controls), 6.4, 13, 26, 50, and 98 mg total radioactive residues (TRR)/kg dw sediment (based upon LSC analysis) |
| <u>Number of replicates:</u> | 12 replicates per level |
| <u>Number of organisms:</u> | 12 chironomids per replicate |
| Test organisms randomly or impartially assigned to test vessels? | Yes |
| Chemical analysis | Sediment, pore water, and overlying water samples were analyzed on days -1, 20, and 54 for total [¹⁴ C]residues (TRR) using LSC. Radioactive residues were not further characterized. |

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| Parameter | Details |
|--|---|
| Interstitial water and sediment isolation method | Centrifugation for 30 minutes at 10,000 g. |
| Recovery of chemical: | Based on QC samples prepared and analyzed concurrently with sample analysis: <u>LSC</u> Sediment: 86.3 to 110% of nominal Overlying water: 96.7 to 107% of nominal |
| Positive control | N/A |
| Other parameters, if any | N/A |

2. Observations:

Table 7: Observations

| Parameters | Details |
|--------------------------------|--|
| Data endpoints measured (list) | <u>Day 20:</u> - Survival - Ash-free dry weight <u>Day 54 (test termination):</u> - Percent emergence - Development rate - Time to death (for mated individuals) - Number of eggs per female - Percent hatch |
| Observation intervals | All vessels were examined daily for survival and abnormal behavior. Reproductive/oviposit chambers were checked daily for dead adults and egg masses and dead flies were removed. Due to poor viability, secondary egg masses (if present) were not included in egg numbers or to determine percent hatch. |
| Were raw data included? | Yes, sufficient. |
| Other observations, if any | N/A |

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II. REPORTED RESULTS:

A. REPORTED EFFECTS ON DAY 20 (SURVIVAL AND GROWTH):

Mean percent survival at 20 days was 88, 94, 94, 96, 92, 48, and 71% in the negative control, solvent control, 6.4, 13, 26, 50, and 98 mg/kg treatment levels, respectively (based upon mean-measured sediment concentrations). The study author reported that differences were statistically-reduced ($p \leq 0.05$) compared to the pooled control (91%) for the 50 and 98 mg/kg sediment levels. The NOAEC for 20-day survival was 26 mg/kg sediment. The 20-day LC₅₀ was estimated to be >98 mg/kg sediment.

Mean ash-free dry weight at 20 days was 1.59, 1.47, 1.48, 1.37, 1.41, 1.40, and 1.18 mg per larvae in the negative control, solvent control, 6.4, 13, 26, 50, and 98 mg/kg treatment levels, respectively. The study author reported that the difference was statistically-significant ($p \leq 0.05$) from the pooled control (1.53 mg) at the 98 mg/kg level, and the resultant NOAEC was 50 mg/kg sediment. The 20-day EC₅₀ was estimated to be >98 mg/kg sediment.

Table 8: Effects of AEC656948 on Survival and Growth of the Midge (*Chironomus tentans*) at Day 20.

| Mean-measured (and Nominal) Sediment Conc., mg/kg dw ^(a) | Time-Weighted Average (TWA) Concentrations ^(b) | | | Day 20 | |
|--|---|-------------------------|---------------------------------|---------------------|---|
| | Sediment, mg TRR/kg | Pore Water, mg TRR/L | Overlying Water, mg TRR/L | Mean Survival, % | Mean Ash-Free Dry Weight per Larvae, mg |
| Control | <0.051 | <0.0093 | <0.0029 | 88 | 1.59 |
| Solvent Control | <0.044 | <0.0097 | <0.0029 | 94 | 1.47 |
| 6.4 (7.5) | 6.2 | 0.66 | 0.032 | 94 | 1.48 |
| 13 (15) | 13 | 1.9 | 0.047 | 96 | 1.37 |
| 26 (30) | 26 | 3.8 | 0.14 | 92 | 1.41 |
| 50 (60) | 48 | 8.8 | 0.29 | 48* | 1.40 |
| 98 (120) | 96 | 18 | 0.88 | 71* | 1.18* |
| NOAEC, mg/kg sediment (mean-measured) | | | | 26 | 50 |
| LOAEC, mg/kg sediment (mean-measured) | | | | 50 | 98 |
| LC/EC ₅₀ , mg/kg sediment (mean-measured) | | | | >98 | >98 |

^(a) Mean-measured sediment concentrations were study-author calculated and represent total [¹⁴C]AEC656948 equivalents.

^(b) Reviewer-calculated using Excel software (refer to Appendix II for copy of Excel worksheet).

*Statistically different ($p \leq 0.05$) compared to the pooled controls based on Bonferroni's Test.

B. REPORTED EFFECTS ON DAY 54 (EMERGENCE, DAYS TO DEATH, AND REPRODUCTION):

Mean percent emergence was 78, 83, 69, 77, 78, 50, and 52% for the control, solvent control, 6.4, 13, 26, 50, and 98 mg/kg treatment levels, respectively (based upon mean-measured sediment concentrations). The study author reported that differences were statistically-reduced ($p \leq 0.05$) compared to the pooled control (81%) for

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the 50 and 98 mg/kg sediment levels. The NOAEC for percent emergence was 26 mg/kg sediment.

Mean development rates (combined sexes) were 0.0392, 0.0401, 0.0455, 0.0361, 0.0396, 0.0369, and 0.0293 day⁻¹ for the control, solvent control, 6.4, 13, 26, 50, and 98 mg/kg treatment levels, respectively. The study author reported that differences were statistically-significant ($p \leq 0.05$) from the pooled control (0.0401 day⁻¹) at the 13 and 98 mg/kg sediment levels; however, the effect observed at the 13 mg/kg level was not considered to be biologically relevant due to a lack of similar response at the 26 and 50 mg/kg sediment levels. Thus, the NOAEC for the combined sex development rate was reported to be 50 mg/kg sediment.

Table 9: Effects of AEC656948 on Emergence and Development of the Midge (*Chironomus tentans*) at Day 54.

| Mean-measured Sediment, mg total residues/kg dw | Day 54 | |
|--|------------------------|---|
| | Mean Percent Emergence | Mean Development Rate (Male/Female Combined) |
| Control | 78 | 0.0392 |
| Solvent Control | 83 | 0.0410 |
| 6.4 | 69 | 0.0455 |
| 13 | 77 | 0.0361 ^{*(a)} |
| 26 | 78 | 0.0396 |
| 50 | 50* | 0.0369 |
| 98 | 52* | 0.0293* |

^(a) Although statistically-significant from the pooled control, this difference was not considered to be biologically relevant due to the lack of a similar response at the next two higher treatment levels.

^(b) Mean development rate per vessel, \bar{x} , is calculated according to:

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$$\bar{x} = \sum_{i=1}^m \frac{f_i x_i}{n_e}$$

where:

- \bar{x} : mean development rate per vessel
- i : index of inspection interval
- m : maximum number of inspection intervals
- f_i : number of midges emerged in the inspection interval i
- n_e : total number of midges emerged at the end of experiment ($= \sum f_i$)
- x_i : development rate of the midges emerged in interval i

$$x_i = \frac{1}{\left(\text{day}_i - \frac{l_i}{2} \right)}$$

where:

- day_i : inspection day (days since application)
- l_i : length of inspection interval i (days, usually 1 day)

*Statistically different ($p \leq 0.05$) compared to the pooled controls based on Bonferroni's Test.

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No statistically-significant differences were indicated on the total mean number of days to death, which ranged from 3.9 to 5.0 days for all control and exposure levels. Similarly, no statistically-significant differences were observed for the mean number of eggs laid per female, which ranged from 796 to 1175 for all control and exposure levels. The NOAEC for both endpoints was 98 mg/kg sediment, the highest mean-measured concentration tested.

The mean percent hatch was 99, 99, 97, 98, 98, 95, 99% for the control, solvent control, 6.4, 13, 26, 50, and 98 mg/kg treatment levels, respectively. The difference was statistically-significant ($p \leq 0.05$) from the pooled control (99%) at the 50 mg/kg sediment level; however, the effect was not considered to be biologically relevant due to a lack of similar response at the 98 mg/kg level. Thus, the NOAEC for percent hatch was reported by the study author to be 98 mg/kg sediment.

Table 10: Effects of AEC656948 on Mean Days to Death and Reproduction of the Midge (*Chironomus tentans*).

| Mean-measured Sediment, mg total residues/kg dw | Mean Days to Death | | | | Mean No. of Total Eggs per Female | Mean Percent Hatch |
|---|--------------------|-------------|---------------|-------|-----------------------------------|--------------------|
| | Unmated Males | Mated Males | Mated Females | Total | | |
| Control | 2.5 | 3.9 | 4.4 | 4.1 | 796 | 99 |
| Solvent Control | 3.3 | 4.1 | 4.4 | 4.3 | 1082 | 99 |
| 6.4 | 2.8 | 3.6 | 4.3 | 3.9 | 1049 | 97 |
| 13 | 2.7 | 4.8 | 4.5 | 4.5 | 960 | 98 |
| 26 | 2.7 | 4.3 | 4.4 | 4.4 | 1038 | 98 |
| 50 | 2.8 | 3.7 | 4.2 | 4.0 | 1175 | 95 ^{*(a)} |
| 98 | 2.4 | 4.6 | 5.1 | 5.0 | 853 | 99 |

^(a) Although statistically-significant, this difference was not considered to be biologically relevant due to the lack of a similar response at the next higher treatment level. Replicate data were not provided for the reviewer to statistically verify results for this endpoint.

C. REPORTED STATISTICS:

Endpoints analyzed were survival and growth (ash-free dry weight) on Day 20; and percent emergence, development rate, time to death of mated individuals, number of eggs per female, and percent hatch of eggs at Day 54. Analyses were performed with TOXSTAT Version 3.5 statistical software using the mean replicate organism response in each treatment group. Percent survival data were arcsine square-root transformed prior to analysis. Data were assessed using mean-measured sediment concentrations.

For all endpoints, a t-Test was conducted to compare the performance of the negative and solvent control organisms. For all endpoints, no differences were observed for any endpoint and the data were pooled for subsequent comparisons. The data were tested for normality using the Shapiro-Wilk's Test (growth data on Day 20) or the Chi-Square Test (all other endpoints), and for homogeneity of variance using Bartlett's Test (all endpoints). Except for percent hatch, all sets of data were normally distributed and met the assumption for homogeneity. Bonferroni's t-Test was used to establish treatment effects for survival, growth, percent emergence, development rate, number of

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eggs per female, and days to death. For percent hatch, the Kruskal-Wallis Test was used to establish treatment effects. NOAEC and LOAEC values were assigned based on significance.

As mortality did not exceed 50% at any concentration level, the LC₅₀ was empirically estimated to be greater than the highest mean-measured sediment concentration tested. Similarly, no concentration tested resulted in a $\geq 50\%$ reduction in 20-day dry weights, and therefore the EC₅₀ for this endpoint was empirically estimated to be greater than the highest mean-measured sediment concentration tested.

| Endpoint | Methods | NOAEC, (mg/kg) | LOAEC, (mg/kg) | LC/EC ₅₀ , (mg/kg) |
|-----------------------|---------------------|-------------------|-------------------|----------------------------------|
| Survival (day 20) | Bonferroni's t-Test | 26 | 50 | >98 |
| Dry weight (day 20) | Bonferroni's t-Test | 50 | 98 | >98 |
| Percent emergence | Bonferroni's t-Test | 26 | 50 | ---- |
| Development rate | Bonferroni's t-Test | 50 | 98 | ---- |
| Time to death | Bonferroni's t-Test | 98 | >98 | ---- |
| Number of eggs/female | Bonferroni's t-Test | 98 | >98 | ---- |
| Percent hatch | Kruskal-Wallis Test | 98 | >98 | ---- |

III. REVIEWER'S EVALUATION:

A. DEVIATIONS FROM GUIDELINES:

1. Guidance specifies that the treatment level (individually if necessary) should be terminated when no further emergence is recorded over a 7-day period. In this study it was reported that minor emergence (1 to 2 individuals per group) was encountered at some treatment levels during the 7-day period prior to study termination on day 54. The study author reported that the duration of the test was appropriate to gather the data needed for the various endpoints assessed.
2. In addition to larval survival on day 20, pupa and adult mortality should also have been evaluated.
3. The mean number of eggs per egg case was 796 for the negative control group, which was slightly less than the acceptability level of 800 eggs per egg case delineated in guidance.

B. OTHER STUDY DEFICIENCIES:

1. Physical descriptions of the test substances were not provided.
2. Pre-test mortality of the newly-hatched larvae should have been reported.
3. Although total [¹⁴C]residues were determined at -1, 20, and 54 days in the overlying water, pore water, and sediment, the radioactivity was not further characterized for possible degradation. Therefore, the stability of the test substance was not investigated. This, however, is not required in U.S. EPA Test Method 100.5.

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C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The reviewer verified the results for day 20 survival and growth (dry weight), percent emergence, and male and female development rate. Data for number of eggs/female could be visually verified, as responses in treated groups were all greater than that in the negative control group. Replicate data for percent hatch were not provided, so this endpoint was visually determined as well. For all statistically analyzed endpoints, the negative control group was compared to the solvent control using a Student's t-test. No significant differences were detected. All data were tested to confirm that they satisfy the assumptions of normality and homogeneity of variances. The NOAEC and LOAEC values were determined using ANOVA, followed by Dunnett's or William's test. All analyses were conducted using Toxstat statistical software.

| Endpoint | Methods | NOAEC, (mg/kg) | LOAEC, (mg/kg) | LC/EC ₅₀ , (mg/kg) |
|-------------------------|------------------|-------------------|-------------------|----------------------------------|
| Survival (day 20) | ANOVA, Dunnett's | 26 | 48 | >96 |
| Dry weight (day 20) | ANOVA, William's | 48 | 96 | >96 |
| Percent emergence | ANOVA, Dunnett's | 26 | 48 | --- |
| Male Development rate | ANOVA, Dunnett's | 48 | 96 | --- |
| Female Development rate | ANOVA, Dunnett's | 48 | 96 | --- |
| Time to death | Visual | 96 | >96 | --- |
| Number of eggs/female | Visual | 96 | >96 | --- |
| Percent hatch | Visual | 96 | >96 | --- |

D. ADDITIONAL REVIEWER COMMENTS:

The reviewer agrees with the NOAEC and LOAEC values concluded by the study author. However, the reviewer separated the analysis of development rate by sex and detected no differences in sensitivity between the two.

Excluding the number of eggs per egg case, all validity requirements were fulfilled. Specifically, for negative control organisms, midge survival (88%) and ash-free dry weight (1.59 mg/larvae) on day 20 were $\geq 70\%$ and ≥ 0.48 mg/larvae, respectively; emergence (78%) was $\geq 50\%$; the time to death for mated males (3.9 days) was < 6.5 days and for mated females (4.4 days) was < 5.1 days; and the mean percent hatch (99%) was $\geq 80\%$.

To verify the accuracy of the dosing procedure, samples of the dosing stock solutions were analyzed for total radioactivity (TRR) using LSC, and samples of dosed sediment were analyzed following 7 days of mixing and equilibration using LSC following combustion. The TRR in the stock solutions averaged 105 to 115% of nominal concentrations, and in the sediment ranged from 90 to 120% of the nominal treatment levels.

TWA concentrations were reviewer-calculated for sediment, pore water, and overlying water (refer to associated Excel worksheet in Appendix II). As TWA concentrations are more indicative of actual concentration levels, they were reported in the Executive Summary and Conclusions sections of the DER. TWA concentrations were calculated using the following equation:

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$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_n}{2}\right)(t_{n-1} - t_n) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C_{TWA} is the time-weighted average concentration,

C_j is the concentration measured at time interval j ($j = 0, 1, 2, \dots, n$)

t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., $t_0 = 0$ hours (test initiation), $t_1 = 24$ hours, $t_2 = 96$ hours).

[¹⁴C]Residues remained predominantly associated with the sediment during the study, as demonstrated by the $\geq 82\%$ mean percent recoveries of TRR in the sediment. The overlying water and pore water concentrations decreased during the exposure period due to the daily replacements of the overlying water.

The limits of detection ranged from 0.027 to 0.051 mg TRR/kg for sediment, 0.0072 to 0.0097 mg TRR/L for pore water, and 0.0028 to 0.0029 mg TRR/L for overlying water.

Definitive test dates were December 14, 2007 to February 5, 2008.

E. CONCLUSIONS:

This study is **scientifically sound/unsound and is thus acceptable or unacceptable**. During a life-cycle toxicity test, exposure to AEC656948 (fluopyram) adversely affected larval survival (day 20) and percent emergence (day 54) of the freshwater dipteran midge (*Chironomus tentans*) at the two highest concentration levels. Larval ash-free dry weight (day 20) and development rate of both sexes (day 54) were also reduced at the highest treatment level. No treatment-related effect on time to death (mated adults), number of eggs per female, or percent hatch of egg masses were indicated.

Based on TWA sediment concentrations (total radioactive residues):

LC₅₀ survival (day 20): >96 mg/kg

NOAEC (survival): 26 mg/kg

LOAEC (survival): 48 mg/kg

EC₅₀ dry weight (day 20): >96 mg/kg

NOAEC (dry weight): 48 mg/kg

LOAEC (dry weight): 96 mg/kg

NOAEC (percent emergence): 26 mg/kg

LOAEC (percent emergence): 48 mg/kg

NOAEC (combined sex development rate): 48 mg/kg

LOAEC (combined sex development rate): 96 mg/kg

NOAEC (time to death for mated adults): 98 mg/kg

LOAEC (time to death for mated adults): >98 mg/kg

NOAEC (number of eggs per female): 98 mg/kg

LOAEC (number of eggs per female): >98 mg/kg

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NOAEC (percent hatch of egg masses): 98 mg/kg
LOAEC (percent hatch of egg masses): >98 mg/kg

Based on TWA pore water concentrations (total radioactive residues):

LC₅₀ survival (day 20): >18 mg/L

NOAEC (survival): 3.8 mg/L

LOAEC (survival): 8.8 mg/L

EC₅₀ dry weight (day 20): >18 mg/L

NOAEC (dry weight): 8.8 mg/L

LOAEC (dry weight): 18 mg/L

NOAEC (percent emergence): 3.8 mg/L

LOAEC (percent emergence): 8.8 mg/L

NOAEC (combined sex development rate): 8.8 mg/L

LOAEC (combined sex development rate): 18 mg/L

NOAEC (time to death for mated adults): 18 mg/L

LOAEC (time to death for mated adults): >18 mg/L

NOAEC (number of eggs per female): 18 mg/L

LOAEC (number of eggs per female): >18 mg/L

NOAEC (percent hatch of egg masses): 18 mg/L

LOAEC (percent hatch of egg masses): >18 mg/L

Based on TWA overlying water concentrations (total radioactive residues):

LC₅₀ survival (day 20): >0.88 mg/L

NOAEC (survival): 0.14 mg/L

LOAEC (survival): 0.29 mg/L

EC₅₀ dry weight (day 20): >0.88 mg/L

NOAEC (dry weight): 0.29 mg/L

LOAEC (dry weight): 0.88 mg/L

NOAEC (percent emergence): 0.14 mg/L

LOAEC (percent emergence): 0.29 mg/L

NOAEC (combined sex development rate): 0.29 mg/L

LOAEC (combined sex development rate): 0.88 mg/L

NOAEC (time to death for mated adults): 0.88 mg/L

LOAEC (time to death for mated adults): >0.88 mg/L

NOAEC (number of eggs per female): 0.88 mg/L

LOAEC (number of eggs per female): >0.88 mg/L

NOAEC (percent hatch of egg masses): 0.88 mg/L

LOAEC (percent hatch of egg masses): >0.88 mg/L

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

day 20 survival

File: 2339 Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

-
GRP1 (SOLVENT CTRL) MEAN = 87.7500 CALCULATED t VALUE = -1.0221
GRP2 (BLANK CTRL) MEAN = 93.7500 DEGREES OF FREEDOM = 6
DIFFERENCE IN MEANS = -6.0000

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at
alpha=0.05

TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at
alpha=0.01

day 20 survival

File: 2339 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL <-1.5 -1.5 to <-0.5 -0.5 to 0.5 >0.5 to 1.5 >1.5

EXPECTED 1.608 5.808 9.168 5.808 1.608
OBSERVED 0 7 9 8 0

Calculated Chi-Square goodness of fit test statistic = 4.2910

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

day 20 survival

File: 2339 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 2665.000

W = 0.913

Critical W (P = 0.05) (n = 24) = 0.916

Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

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day 20 survival

File: 2339

Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 17.89

Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3

Actual values ==> R (# groups) = 6, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

day 20 survival

File: 2339

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 7.81

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

day 20 survival

File: 2339

Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|----------|----------|-------|
| Between | 5 | 6968.333 | 1393.667 | 9.413 |
| Within (Error) | 18 | 2665.000 | 148.056 | |
| Total | 23 | 9633.333 | | |

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Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho:All groups equal

day 20 survival
File: 2339 Transform: NO TRANSFORMATION

| DUNNETTS TEST - TABLE 1 OF 2 | | Ho:Control<Treatment | | | |
|------------------------------|----------------|----------------------|-----------------------------------|--------|-----|
| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
| 1 | neg control | 87.750 | 87.750 | | |
| 2 | 6.2 | 93.750 | 93.750 | -0.697 | |
| 3 | 13 | 96.000 | 96.000 | -0.959 | |
| 4 | 26 | 91.750 | 91.750 | -0.465 | |
| 5 | 48 | 48.000 | 48.000 | 4.620 | * |
| 6 | 96 | 70.750 | 70.750 | 1.976 | |

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

day 20 survival
File: 2339 Transform: NO TRANSFORMATION

| DUNNETTS TEST - TABLE 2 OF 2 | | Ho:Control<Treatment | | | |
|------------------------------|----------------|----------------------|-----------------------------------|--------------|-------------------------|
| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
| 1 | neg control | 4 | | | |
| 2 | 6.2 | 4 | 20.736 | 23.6 | -6.000 |
| 3 | 13 | 4 | 20.736 | 23.6 | -8.250 |
| 4 | 26 | 4 | 20.736 | 23.6 | -4.000 |
| 5 | 48 | 4 | 20.736 | 23.6 | 39.750 |
| 6 | 96 | 4 | 20.736 | 23.6 | 17.000 |

day 20 survival
File: 2339 Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2 | | | | | |
|--|----------------|---|---------------|------------------|-----------------|
| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
| 1 | neg control | 4 | 87.750 | 87.750 | 92.500 |
| 2 | 6.2 | 4 | 93.750 | 93.750 | 92.500 |
| 3 | 13 | 4 | 96.000 | 96.000 | 92.500 |
| 4 | 26 | 4 | 91.750 | 91.750 | 91.750 |

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| | | | | | |
|---|----|---|--------|--------|--------|
| 5 | 48 | 4 | 48.000 | 48.000 | 59.375 |
| 6 | 96 | 4 | 70.750 | 70.750 | 59.375 |

day 20 survival

File: 2339

Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) | | | | TABLE 2 OF 2 | |
|---|-----------------|----------------|-----------|----------------|--------------------|
| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
| neg control | 92.500 | | | | |
| 6.2 | 92.500 | 0.552 | | 1.73 | k= 1, v=18 |
| 13 | 92.500 | 0.552 | | 1.82 | k= 2, v=18 |
| 26 | 91.750 | 0.465 | | 1.85 | k= 3, v=18 |
| 48 | 59.375 | 3.298 | * | 1.86 | k= 4, v=18 |
| 96 | 59.375 | 3.298 | * | 1.87 | k= 5, v=18 |

s = 12.168

Note: df used for table values are approximate when v > 20.

ash-free dry weight per midge larvae (mg)

File: 2339w

Transform: NO TRANSFORM

| t-test of Solvent and Blank Controls | | | | Ho:GRP1 MEAN = GRP2 MEAN | |
|---|--------|----------------------|--------|--------------------------|--|
| GRP1 (SOLVENT CRTL) MEAN = | 1.5875 | CALCULATED t VALUE = | 0.9767 | | |
| GRP2 (BLANK CRTL) MEAN = | 1.4650 | DEGREES OF FREEDOM = | 6 | | |
| DIFFERENCE IN MEANS = | 0.1225 | | | | |
| TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05 | | | | | |
| TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01 | | | | | |

ash-free dry weight per midge larvae (mg)

File: 2339w

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

| INTERVAL | <-1.5 | -1.5 to <-0.5 | -0.5 to 0.5 | >0.5 to 1.5 | >1.5 |
|----------|-------|---------------|-------------|-------------|-------|
| EXPECTED | 1.608 | 5.808 | 9.168 | 5.808 | 1.608 |
| OBSERVED | 0 | 6 | 10 | 8 | 0 |

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Calculated Chi-Square goodness of fit test statistic = 4.1251
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

ash-free dry weight per midge larvae (mg)
File: 2339w Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.930

W = 0.966

Critical W (P = 0.05) (n = 24) = 0.916

Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

ash-free dry weight per midge larvae (mg)
File: 2339w Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 3.60
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

ash-free dry weight per midge larvae (mg)
File: 2339w Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 1.55

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Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

ash-free dry weight per midge larvae (mg)

File: 2339w Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|-------|-------|-------|
| Between | 5 | 0.359 | 0.072 | 1.385 |
| Within (Error) | 18 | 0.930 | 0.052 | |
| Total | 23 | 1.288 | | |

Critical F value = 2.77 (0.05,5,18)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

ash-free dry weight per midge larvae (mg)

File: 2339w Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|----------------|------------------|-----------------------------------|--------|-----|
| 1 | neg control | 1.587 | 1.587 | | |
| 2 | 6.2 | 1.478 | 1.478 | 0.682 | |
| 3 | 13 | 1.365 | 1.365 | 1.380 | |
| 4 | 26 | 1.407 | 1.407 | 1.116 | |
| 5 | 48 | 1.400 | 1.400 | 1.163 | |
| 6 | 96 | 1.183 | 1.183 | 2.512 | * |

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

ash-free dry weight per midge larvae (mg)

File: 2339w Transform: NO TRANSFORMATION

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| DUNNETTS TEST | | - TABLE 2 OF 2 | | Ho:Control<Treatment | | |
|---------------|----------------|----------------|-----------------------------------|----------------------|-------------------------|--|
| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL | |
| 1 | neg control | 4 | | | | |
| 2 | 6.2 | 4 | 0.389 | 24.5 | 0.110 | |
| 3 | 13 | 4 | 0.389 | 24.5 | 0.222 | |
| 4 | 26 | 4 | 0.389 | 24.5 | 0.180 | |
| 5 | 48 | 4 | 0.389 | 24.5 | 0.188 | |
| 6 | 96 | 4 | 0.389 | 24.5 | 0.405 | |

ash-free dry weight per midge larvae (mg)

File: 2339w Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) | | TABLE 1 OF 2 | | | |
|---|----------------|--------------|---------------|------------------|-----------------|
| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
| 1 | neg control | 4 | 1.587 | 1.587 | 1.587 |
| 2 | 6.2 | 4 | 1.478 | 1.478 | 1.478 |
| 3 | 13 | 4 | 1.365 | 1.365 | 1.391 |
| 4 | 26 | 4 | 1.407 | 1.407 | 1.391 |
| 5 | 48 | 4 | 1.400 | 1.400 | 1.391 |
| 6 | 96 | 4 | 1.183 | 1.183 | 1.183 |

ash-free dry weight per midge larvae (mg)

File: 2339w Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) | | TABLE 2 OF 2 | | | |
|---|-----------------|----------------|-----------|----------------|--------------------|
| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
| neg control | 1.587 | | | | |
| 6.2 | 1.478 | 0.685 | | 1.73 | k= 1, v=18 |
| 13 | 1.391 | 1.224 | | 1.82 | k= 2, v=18 |
| 26 | 1.391 | 1.224 | | 1.85 | k= 3, v=18 |
| 48 | 1.391 | 1.224 | | 1.86 | k= 4, v=18 |
| 96 | 1.183 | 2.520 | * | 1.87 | k= 5, v=18 |

s = 0.227

Note: df used for table values are approximate when v > 20.

percent emerged

File: 2339e Transform: NO TRANSFORM

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

EPA MRID No. 473723-39

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN = 78.1246 CALCULATED t VALUE = -1.2577
 GRP2 (BLANK CTRL) MEAN = 83.3400 DEGREES OF FREEDOM = 14
 DIFFERENCE IN MEANS = -5.2154

TABLE t VALUE (0.05 (2),14) = 2.145 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2),14) = 2.977 NO significant difference at alpha=0.01

percent emerged
 File: 2339e Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

| INTERVAL | <-1.5 | -1.5 to <-0.5 | -0.5 to 0.5 | >0.5 to 1.5 | >1.5 |
|----------|-------|---------------|-------------|-------------|-------|
| EXPECTED | 3.216 | 11.616 | 18.336 | 11.616 | 3.216 |
| OBSERVED | 3 | 11 | 16 | 16 | 2 |

Calculated Chi-Square goodness of fit test statistic = 2.4591
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

percent emerged
 File: 2339e Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 12103.861

W = 0.947

Critical W (P = 0.05) (n = 48) = 0.947
 Critical W (P = 0.01) (n = 48) = 0.929

Data PASS normality test at P=0.01 level. Continue analysis.

percent emerged
 File: 2339e Transform: NO TRANSFORMATION

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

EPA MRID No. 473723-39

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 15.36
Closest, conservative, Table H statistic = 18.4 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 7
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 7.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

percent emerged
File: 2339e Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 14.09
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 7.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

percent emerged
File: 2339e Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE DF SS MS F

Between 5 6905.317 1381.063 4.792
Within (Error) 42 12103.861 288.187

Total 47 19009.178

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

EPA MRID No. 473723-39

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

percent emerged
File: 2339e Transform: NO TRANSFORMATION

| DUNNETTS TEST - TABLE 1 OF 2 | | Ho:Control<Treatment | | | |
|------------------------------|----------------|----------------------|-----------------------------------|--------|-----|
| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
| 1 | neg control | 78.125 | 78.125 | | |
| 2 | 6.2 | 68.758 | 68.758 | 1.104 | |
| 3 | 13 | 77.097 | 77.097 | 0.121 | |
| 4 | 26 | 78.113 | 78.113 | 0.001 | |
| 5 | 48 | 49.996 | 49.996 | 3.314 | * |
| 6 | 96 | 52.083 | 52.083 | 3.068 | * |

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

percent emerged
File: 2339e Transform: NO TRANSFORMATION

| DUNNETTS TEST - TABLE 2 OF 2 | | Ho:Control<Treatment | | | |
|------------------------------|----------------|----------------------|-----------------------------------|--------------|-------------------------|
| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
| 1 | neg control | 8 | | | |
| 2 | 6.2 | 8 | 19.607 | 25.1 | 9.367 |
| 3 | 13 | 8 | 19.607 | 25.1 | 1.028 |
| 4 | 26 | 8 | 19.607 | 25.1 | 0.012 |
| 5 | 48 | 8 | 19.607 | 25.1 | 28.129 |
| 6 | 96 | 8 | 19.607 | 25.1 | 26.042 |

percent emerged
File: 2339e Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2 | | | | | |
|--|----------------|---|---------------|------------------|-----------------|
| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
| 1 | neg control | 8 | 78.125 | 78.125 | 78.125 |
| 2 | 6.2 | 8 | 68.758 | 68.758 | 74.656 |
| 3 | 13 | 8 | 77.097 | 77.097 | 74.656 |
| 4 | 26 | 8 | 78.113 | 78.113 | 74.656 |
| 5 | 48 | 8 | 49.996 | 49.996 | 51.039 |
| 6 | 96 | 8 | 52.083 | 52.083 | 51.039 |

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

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percent emerged

File: 2339e

Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) | | | | TABLE 2 OF 2 | |
|---|-----------------|----------------|-----------|----------------|--------------------|
| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
| neg control | 78.125 | | | | |
| 6.2 | 74.656 | 0.409 | | 1.68 | k= 1, v=42 |
| 13 | 74.656 | 0.409 | | 1.76 | k= 2, v=42 |
| 26 | 74.656 | 0.409 | | 1.79 | k= 3, v=42 |
| 48 | 51.039 | 3.191 | * | 1.80 | k= 4, v=42 |
| 96 | 51.039 | 3.191 | * | 1.80 | k= 5, v=42 |

s = 16.976

Note: df used for table values are approximate when v > 20.

male development rate

File: 2339md

Transform: NO TRANSFORM

| t-test of Solvent and Blank Controls | | | Ho:GRP1 MEAN = GRP2 MEAN | |
|---|---------|----------------------|--------------------------|--|
| GRP1 (SOLVENT CRTL) MEAN = | 4.1550 | CALCULATED t VALUE = | -1.2076 | |
| GRP2 (BLANK CRTL) MEAN = | 4.4631 | DEGREES OF FREEDOM = | 14 | |
| DIFFERENCE IN MEANS = | -0.3081 | | | |
| TABLE t VALUE (0.05 (2),14) = 2.145 NO significant difference at alpha=0.05 | | | | |
| TABLE t VALUE (0.01 (2),14) = 2.977 NO significant difference at alpha=0.01 | | | | |

male development rate

File: 2339md

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

| INTERVAL | <-1.5 | -1.5 to <-0.5 | -0.5 to 0.5 | >0.5 to 1.5 | >1.5 |
|----------|-------|---------------|-------------|-------------|-------|
| EXPECTED | 3.216 | 11.616 | 18.336 | 11.616 | 3.216 |
| OBSERVED | 5 | 7 | 21 | 13 | 2 |

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

EPA MRID No. 473723-39

Calculated Chi-Square goodness of fit test statistic = 3.8357
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

male development rate
File: 2339md Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 14.412

W = 0.837

Critical W (P = 0.05) (n = 48) = 0.947
Critical W (P = 0.01) (n = 48) = 0.929

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

male development rate
File: 2339md Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 9.95
Closest, conservative, Table H statistic = 18.4 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 7
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 7.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

male development rate
File: 2339md Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

EPA MRID No. 473723-39

Calculated B statistic = 15.50
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 7.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

male development rate
File: 2339md Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|----------------|----|--------|-------|--------|
| Between | 5 | 20.677 | 4.135 | 12.055 |
| Within (Error) | 42 | 14.412 | 0.343 | |
| Total | 47 | 35.090 | | |

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

male development rate
File: 2339md Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|----------------|------------------|-----------------------------------|--------|-----|
| 1 | neg control | 4.155 | 4.155 | | |
| 2 | 6.2 | 4.800 | 4.800 | -2.203 | |
| 3 | 13 | 3.787 | 3.787 | 1.255 | |
| 4 | 26 | 4.088 | 4.088 | 0.231 | |
| 5 | 48 | 3.813 | 3.813 | 1.170 | |
| 6 | 96 | 2.613 | 2.613 | 5.268 | * |

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

male development rate
File: 2339md Transform: NO TRANSFORMATION

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

EPA MRID No. 473723-39

| DUNNETTS TEST - TABLE 2 OF 2 | | | Ho:Control<Treatment | | |
|------------------------------|----------------|-------------|-----------------------------------|--------------|-------------------------|
| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
| 1 | neg control | 8 | | | |
| 2 | 6.2 | 8 | 0.676 | 16.3 | -0.645 |
| 3 | 13 | 8 | 0.676 | 16.3 | 0.368 |
| 4 | 26 | 8 | 0.676 | 16.3 | 0.067 |
| 5 | 48 | 8 | 0.676 | 16.3 | 0.342 |
| 6 | 96 | 8 | 0.676 | 16.3 | 1.542 |

male development rate

File: 2339md Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) | | | TABLE 1 OF 2 | | |
|---|----------------|---|---------------|------------------|-----------------|
| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
| 1 | neg control | 8 | 4.155 | 4.155 | 4.478 |
| 2 | 6.2 | 8 | 4.800 | 4.800 | 4.478 |
| 3 | 13 | 8 | 3.787 | 3.787 | 3.938 |
| 4 | 26 | 8 | 4.088 | 4.088 | 3.938 |
| 5 | 48 | 8 | 3.813 | 3.813 | 3.813 |
| 6 | 96 | 8 | 2.613 | 2.613 | 2.613 |

male development rate

File: 2339md Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) | | | TABLE 2 OF 2 | | |
|---|-----------------|----------------|--------------|----------------|--------------------|
| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
| neg control | 4.478 | | | | |
| 6.2 | 4.478 | 1.101 | | 1.68 | k= 1, v=42 |
| 13 | 3.938 | 0.743 | | 1.76 | k= 2, v=42 |
| 26 | 3.938 | 0.743 | | 1.79 | k= 3, v=42 |
| 48 | 3.813 | 1.169 | | 1.80 | k= 4, v=42 |
| 96 | 2.613 | 5.266 | * | 1.80 | k= 5, v=42 |

s = 0.586

Note: df used for table values are approximate when v > 20.

female development rate

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

EPA MRID No. 473723-39

File: 2339fd Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN = 3.6750 CALCULATED t VALUE = -0.6487
GRP2 (BLANK CTRL) MEAN = 3.8375 DEGREES OF FREEDOM = 14
DIFFERENCE IN MEANS = -0.1625

TABLE t VALUE (0.05 (2),14) = 2.145 NO significant difference at
alpha=0.05
TABLE t VALUE (0.01 (2),14) = 2.977 NO significant difference at
alpha=0.01

female development rate

File: e Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL <-1.5 -1.5 to <-0.5 -0.5 to 0.5 >0.5 to 1.5 >1.5

EXPECTED 3.216 11.616 18.336 11.616 3.216
OBSERVED 6 7 14 20 1

Calculated Chi-Square goodness of fit test statistic = 12.8479
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

female development rate

File: e Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 25.146

W = 0.910

Critical W (P = 0.05) (n = 48) = 0.947
Critical W (P = 0.01) (n = 48) = 0.929

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and
should not be performed.

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

EPA MRID No. 473723-39

female development rate

File: e Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 11.82

Closest, conservative, Table H statistic = 18.4 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 7

Actual values ==> R (# groups) = 6, df (# avg reps-1) = 7.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

female development rate

File: e Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 16.49

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 7.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

female development rate

File: e Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE DF SS MS F

Between 5 19.691 3.938 6.574
Within (Error) 42 25.146 0.599

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

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Total 47 44.837

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

female development rate
File: e Transform: NO TRANSFORMATION

| DUNNETTS TEST - TABLE 1 OF 2 | | Ho:Control<Treatment | | | |
|------------------------------|----------------|----------------------|-----------------------------------|--------|-----|
| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
| 1 | neg control | 3.675 | 3.675 | | |
| 2 | 6.2 | 4.191 | 4.191 | -1.334 | |
| 3 | 13 | 3.513 | 3.513 | 0.420 | |
| 4 | 26 | 3.900 | 3.900 | -0.581 | |
| 5 | 48 | 3.563 | 3.563 | 0.291 | |
| 6 | 96 | 2.162 | 2.162 | 3.909 | * |

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

female development rate
File: e Transform: NO TRANSFORMATION

| DUNNETTS TEST - TABLE 2 OF 2 | | Ho:Control<Treatment | | | |
|------------------------------|----------------|----------------------|-----------------------------------|--------------|-------------------------|
| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
| 1 | neg control | 8 | | | |
| 2 | 6.2 | 8 | 0.894 | 24.3 | -0.516 |
| 3 | 13 | 8 | 0.894 | 24.3 | 0.163 |
| 4 | 26 | 8 | 0.894 | 24.3 | -0.225 |
| 5 | 48 | 8 | 0.894 | 24.3 | 0.113 |
| 6 | 96 | 8 | 0.894 | 24.3 | 1.513 |

female development rate
File: e Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2 | | | | | |
|--|----------------|---|---------------|------------------|-----------------|
| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
| 1 | neg control | 8 | 3.675 | 3.675 | 3.933 |
| 2 | 6.2 | 8 | 4.191 | 4.191 | 3.933 |

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

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| | | | | | |
|---|----|---|-------|-------|-------|
| 3 | 13 | 8 | 3.513 | 3.513 | 3.706 |
| 4 | 26 | 8 | 3.900 | 3.900 | 3.706 |
| 5 | 48 | 8 | 3.563 | 3.563 | 3.563 |
| 6 | 96 | 8 | 2.162 | 2.162 | 2.162 |

female development rate

File: e Transform: NO TRANSFORMATION

| WILLIAMS TEST (Isotonic regression model) | | | | TABLE 2 OF 2 | |
|---|-----------------|----------------|-----------|----------------|--------------------|
| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
| neg control | 3.933 | | | | |
| 6.2 | 3.933 | 0.667 | | 1.68 | k= 1, v=42 |
| 13 | 3.706 | 0.081 | | 1.76 | k= 2, v=42 |
| 26 | 3.706 | 0.081 | | 1.79 | k= 3, v=42 |
| 48 | 3.563 | 0.291 | | 1.80 | k= 4, v=42 |
| 96 | 2.162 | 3.909 | * | 1.80 | k= 5, v=42 |

s = 0.774

Note: df used for table values are approximate when v > 20.

APPENDIX II: COPY OF REVIEWER'S TWA CALCULATIONS:

| SEDIMENT | | | | |
|----------------------------------|------------|--------------------------------|-------------|--|
| Nominal Concentration (mg ai/kg) | Time (Day) | Measured Concentration (mg/kg) | TWA (mg/kg) | |
| 7.5 | -1 | 7.5 | 6.2 | |
| | 20 | 5.3 | | |
| | 54 | 6.4 | | |
| 15.0 | -1 | 14 | 13 | |
| | 20 | 14 | | |
| | 54 | 11 | | |
| 30.0 | -1 | 32 | 26 | |
| | 20 | 24 | | |
| | 54 | 23 | | |
| 60.0 | -1 | 67 | 48 | |
| | 20 | 43 | | |
| | 54 | 40 | | |

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

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| | | | |
|----------------------------------|----------------|-------------------------------|--------------|
| 120.0 | -1 20 54 | 120 100 69 | 96 |
| PORE WATER | | | |
| Nominal Concentration (mg ai/kg) | Time (Day) | Measured Concentration (mg/L) | TWA (mg/L) |
| 7.5 | -1 20 54 | 1.1 0.57 0.49 | 0.66 |
| 15.0 | -1 20 54 | 2.8 1.9 1.2 | 1.9 |
| 30.0 | -1 20 54 | 5.8 3.6 2.8 | 3.8 |
| 60.0 | -1 20 54 | 14.0 8.4 5.6 | 8.8 |
| 120.0 | -1 20 54 | 21 19 13 | 18 |
| OVERLYING WATER | | | |
| Nominal Concentration (mg ai/kg) | Time (Day) | Measured Concentration (mg/L) | TWA (mg/L) |
| 7.5 | -1 20 54 | 0.063 0.033 0.0083 | 0.032 |
| 15.0 | -1 20 54 | 0.12 0.04 0.012 | 0.047 |

Data Summary and Review on the Life-Cycle Toxicity of AEC656948 (Fluopyram) to Freshwater Invertebrates – *Chironomus tentans*

PMRA Submission Number {.....}

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| | | | |
|-------|----|-------|-------------|
| 30.0 | -1 | 0.29 | 0.14 |
| | 20 | 0.14 | |
| | 54 | 0.028 | |
| 60.0 | -1 | 0.57 | 0.29 |
| | 20 | 0.31 | |
| | 54 | 0.062 | |
| 120.0 | -1 | 0.93 | 0.88 |
| | 20 | 1.3 | |
| | 54 | 0.13 | |